11. (new) The method according to claim 5, wherein said $CD34^-$ fraction is frozen after being contacted with said L-leucyl-L-leucine methyl ester.

Remarks

Claims 1-5 are pending in the application. Claims 1 and 4 have been cancelled without prejudice. Claims 2, 3, and 5 have been amended. In amended claims 2, 3, and 5, the acronyms GVHD, DLI, LLME, and HSC, have been replaced with the full terms represented by the acronyms, which are graft versus host disease, donor lymphocyte infusion, L-leucyl-L-leucine methyl ester, and hematopoietic stem cell, respectively. Support for amendment of the acronyms is found on pages 1 and 2. Support for amended claim 2 is found on page 2, line 8, page 5, lines 15-16 and pages 7-10. Support for amended claim 3 is found on pages 7-10. Support for amended claim 5 is found on page 4, lines 8-9, Figures 1 and 2, and page 6, line 24 to page 8, line 6. Support for newly added claim 6 can be found on page 5, lines 11-20. Support for new claims 7 and 8 is found on page 8, lines 27-29 and in Figures 1 and 2. Support for new claim 9 is found on page 6, lines 16-17 and in Figures 1 and 2. Support for new claim 10 is on page 7, line 11 and for new claim 11 it is on page 8, lines 25-27. A "marked up" version of the amended claims is included herein as Appendix A, as required by 37 C.F.R. 1.121(c)(1)(ii).

Declarations

The Examiner asserts that the Declaration as filed is defective because it does not include the mailing address nor residences of the inventors. Please find submitted herewith a properly executed declaration which includes the addresses and residences of the inventors.

Drawings

The Examiner has required that the color drawings will be accepted only if the conditions for accepting color drawings have been satisfied under 37 C.F.R. 1.67(a). Applicants are furnishing substitute black and white drawings herewith to replace the color drawings. The drawings consist of two graphs, Figures 1 and 2, which are being supplied as black and white drawings instead of color. These drawings do not represent new subject

matter. The drawings also comply with the Draftsperson's Notice. Therefore, Applicants respectfully submit that the requirement for correction of color drawings is now moot.

Objections to claims 1-5

Claims 1-5 are objected to because of informalities. Claims 1 and 4 have been cancelled, and therefore the objection as to these claims is moot.

The Examiner asserts that claims 1 and 5 recite "T-cells," whereas claim 2 recites "T cell." The Examiner further asserts that "T cell" is proper. However, the Examiner requests that Applicants choose one form for consistent recitation. Applicants respectfully submit that they do not understand the objection by the Examiner. The use of "T-cells" in claim 5 provides for proper syntax because the plural of T cell is grammatically correct in this instance and one of ordinary skill in the art would recognize the step of "selectively eliminating cytotoxic T-cells" would include more than one T-cell. Furthermore, revising claim 5 to recite that "**a** cytotoxic T-cell" would be selectively eliminated would be inconsistent with the invention and would place an undue limitation on the claim. Applicants also submit that the phrase "following allogeneic T cell-depleted HSCT," as used in claim 2, is grammatically correct. "T cell" as used in claim 2 is part of the adjectival phrase "T cell-depleted" and one of ordinary skill in the art would appreciate its meaning based on the context in which it is used. Applicants respectfully submit that proper syntax is provided in the use of "T-cell" in both claims. Therefore, Applicants request that this objection be withdrawn.

The Examiner has objected to the lack of the use of a colon following the word "comprising" in claims 1 and 5. Claim 1 has been cancelled and claim 5 has been amended by adding a colon after "comprising."

The Examiner has also objected to claim 5 for lacking a semi-colon at the end of part c. The claim has been amended by adding a semicolon to the end of part c, which due to other amendments to the claim described below, is now part d.

Response to the 35 U.S.C. § 112, 2nd paragraph rejection

Claims 1-5 stand rejected under 35 U.S.C. § 112, 2nd paragraph, as allegedly being indefinite.

Claims 1 and 5 stand rejected as being vague and indefinite. It is the opinion of the Examiner that the phrase "eliminating selective cytotoxic T-cells" is unclear as to what a selective cytotoxic T-cell is, and what it is not. Claims 1 and 4 have been cancelled, therefore the rejection as to these claims is moot. Although not necessarily agreeing with the reasoning of the Examiner, in an effort to expedite prosecution of the application, Applicants have amended the phrase "eliminating selective cytotoxic T-cells" to state "selectively eliminating cytotoxic T-cells." Support for this amendment is found on page 4, lines 8-9. Furthermore, the phrase has been amended for use in amended claims 2 and 3, which depended from claim 1, and have been amended to incorporate the subject matter of claim 1.

Claim 2 stands rejected as being nonsensical. In the opinion of the Examiner, claim 2 is nonsensical because it recites the need for one action to follow another, but does not recite the action properly. In an effort to expedite prosecution of the application, Applicants have amended claim 2 to recite the action "HSCT," the acronym for "hematopoietic stem cell transplantation," instead of "HSC," the acronym for "hematopoietic stem cell." Support for this amendment is found on page 2, line 8, and on page 5, lines 15-16. In addition, claims 2 and 3 have been amended to incorporate the subject matter of claim 1, and are now independent claims. The amendment of claims 2 and 3 introduces no new subject matter.

Claim 5 stands rejected as indefinite. In the Examiner's opinion "the HSC" of step a) in line 4 of claim 5 has no antecedent basis. In an effort to expedite prosecution of the application, Applicants have amended claim 5 by adding a new step a), "obtaining a preparation of HSC from a mammal," to provide antecedent basis for "the HSC." This amendment introduces no new subject matter and support for this amendment can be found throughout the application, particularly in Figures 1 and 2, and page 6, line 24 to page 8, line 6. In addition, former step a) is now step b). The amendment of claim 5 introduces no new subject matter.

Applicants believe that the claim amendments and arguments presented above address all issues raised in the Office Action with regard to the 35 U.S.C. § 112, second paragraph rejection of claims 1-5, and that all pending claims are definite. Applicants respectfully request that the 35 U.S.C. § 112, second paragraph rejection be withdrawn.

Benefit of Priority

The Examiner, at page 4 of the Office Action, has denied the benefit of priority of the filing date of U.S. Provisional Application No. 60/188,391, filed March 10, 2000. It is the opinion of the Examiner that there are significant differences between the disclosures of the instant application and '391. One example asserted as evidence of a significant difference by the Examiner is that the '391 application discloses only a method of "preventing GVHD", whereas the instant application recites claims drawn to "the inhibiting of GVHD." The other example asserted by the Examiner is that the '391 application fails to disclose the separation step a) of claim 5.

Applicants respectfully disagree that there are significant differences between '391 and the instant application and submit that the two examples provided by the Examiner are not accurate.

Regarding use of the term "preventing" and the term "inhibiting," Applicants respectfully submit that the terms can be used interchangeably and one of ordinary skill in the art would understand the scope and use of the terms based on the specification as filed. For example, Webster's Third New International Dictionary (1993), Merriam Webster, Inc., Springfield, MA, defines "inhibit" as "to retard or prevent." Thus, as used in '391 and in the instant application, "preventing GVHD" and "inhibiting GVHD" are not significantly different and can be used interchangeably.

Contrary to the assertion of the Examiner, Applicants respectfully submit that the separation step a) of claim 5 is disclosed in '391. The separation step a) of claim 5, "separating the HSC to be infused into $CD34^+$ and $CD34^-$ fractions," is disclosed in '391 at page 2, lines 8-12 where it is stated:

> Another aspect of the present invention is a method of preventing GVHD in a mammal requiring transplant of $CD34^+$ stem cells, comprising **administration of a therapeutically effective amount of LLME to $CD34^-$ PBMC** ex vivo prior to **co-administration** of said **$CD34^-$ PBMC** treated with LLME and **$CD34^+$** stem cells in said mammal requiring transplant of $CD34^+$ stem cells.

(emphasis added). Thus, the statement in '391 describes the use of two different populations of cells derived from a population of hematopoietic stem cells (HSC). Although '391 does not specifically recite the act of "separating" the cells, the act is implied in using the two populations or fractions of cells, and one of ordinary skill in the art would understand that

the act of separating the cells would be required to obtain the two fractions of cells from the parent HSC population. Otherwise, it would not be possible to perform the step of ". . . **co-administration** of said **CD34$^-$ PBMC** treated with LLME and **CD34$^+$** stem cells in said mammal requiring transplant of CD34$^+$ stem cells," recited in '391, because the CD34$^-$ cell population is treated with LLME and the CD34$^+$ stem cells are not. Further support is found in claim 3 of '391 which recites co-administration of the two populations of cells as well. Therefore, the use of the two populations is fully disclosed in the parent '391 application.

In view of the present specification, the context in which the cell populations are described, and the prior art usage as discussed above, one of ordinary skill in the art would readily understand that the scope of stating that using two population of cells, wherein one population is treated and the other is not, would require separating those cells from the heterogeneous cell population source.

Based on the details outlined above, applicants respectfully submit that the instant application does not differ significantly from '391, and request that the denial of benefit of priority to '391 be withdrawn.

Response to the 35 U.S.C. § 102(b) rejection

Claims 1 and 4 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Rosenfeld et al (Transplantation, 1995, 60:7:678-683). The Examiner asserts that Rosenfeld teaches a method of inhibiting graft versus host disease (GVHD) in a human requiring donor lymphocyte infusion (DLI), comprising contacting donor lymphocytes to be infused with an aqueous solution containing a therapeutically effective amount of L-leucyl-L-leucine methyl ester (LLME) ex vivo, to eliminate cytotoxic T cells, and then infusing the lymphocytes and inhibiting GVHD. Claims 1 and 4 have been cancelled herein, therefore the rejection as to these claims is moot. The subject matter of claim 1 has been incorporated into its dependent claims, claims 2 and 3, whose limitations are not anticipated by Rosenfeld et al. Applicants respectfully submit that the rejection under 35 U.S.C. § 102(b) is moot and should be withdrawn.

Response to the 35 U.S.C. § 103(a) rejection

Claims 1 and 3-5 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Rosenfeld in view of Small et al. (Blood, 1999, 93:467-480) and U.S. Patent No. 5,668,112

to Lipsky et al. Claims 1 and 4 have been cancelled herein, and the rejection as to these claims is moot. In the view of the Examiner, Rosenfeld teaches that, in addition to the above-described teachings, many stem cell transplantation patients suffer and die from infections. The Examiner also asserts that Rosenfeld teaches that LLME treatment may cause some reduction in CFU-GM. The Examiner does admit, however, that Rosenfeld differs from the claimed invention in that Rosenfeld does not teach the use of DLI after donor stem cell engraftment or the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME.

It is the view of the Examiner that Small et al. teaches that the risk of opportunistic infection in stem cell transplant patients is inversely proportional to the levels of CD4 T cells. It is also the view of the Examiner that '112 teaches that NK cells and cytotoxic T cells are primarily responsible for GVHD after DLI and that ex vivo LLME treatment can be used to kill these cells before DLI.

The Examiner asserts at page 6 of the Office Action that it would have been *prima facie* obvious to one of ordinary skill in the art at the time that invention was made to infuse LLME-treated donor lymphocytes into a mammal after stem cell transplantation, in view of the combined teachings of Rosenfeld, Small, and the '756 patent. Applicants assume that the Examiner meant to cite the "'112" patent and not a "'756 patent," because the Examiner cited the '112 patent in the first paragraph of the 103(a) rejection and elsewhere in the 103(a) rejection, and a '756 patent is not cited anywhere else in the Office Action. Therefore, Applicants are responding to the rejection based on the '112 patent. The Examiner further asserts that one of ordinary skill in the art at the time that the invention was made would have been motivated to infuse LLME-treated donor lymphocytes into a mammal after stem cell transplantation because the mammals would be in need of the additional infusion to fight opportunistic infections, as taught by Rosenfeld et al. The Examiner then suggests that, given that low CD4 T cell counts correlate with an increased risk of infection, as taught by Small, and increased CD8 and NK cell counts increase the risk of GVHD, as taught by the '112 patent, one of ordinary skill in the art at the time the invention was made would have been motivated to use LLME-treated donor lymphocytes because said lymphocytes would have been increased in CD4 cells, and that in particular, the increase would have been achieved without an additional increase in the depleted CD8

and NK cells, and would thus, have increased the ability to inhibit opportunistic infection without increasing the risk of GVHD.

Claim 5 stands rejected because it is the opinion of the Examiner that it would have been obvious in view of the combined references to separate the cells for transplantation into CD34+ (stem cell) and CD34- (nonstem cell) fractions before LLME treatment, and treat only the CD34- fraction, while leaving the CD34+ fraction (the fraction comprising the stem cells that differentiate into CFU-GM) untreated, thus obtaining the benefits of NK and cytotoxic T cell reduction, without concurrent CFU-GM reduction.

Applicants respectfully submit that the combination of Rosenfeld, Small, and '112 does not render claims 3 and 5 *prima facie* obvious under 35 U.S.C. § 103(a), claims 1 and 4 having been cancelled, for the following reasons.

Preliminarily, the three-prong test which must be met for a reference or a combination of references to establish a prima facie case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

To support a case of *prima facie* obviousness, a combination of references must: (1) suggest to those of ordinary skill in the art that they should make the claimed invention, and (2) reveal to those of ordinary skill in the art that they would have a reasonable expectation of success. In re Vaeck, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be found in the prior art and not in Applicant's disclosure. In re Dow Chemical Company, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). None of these criteria have been met here.

Amended claim 3 recites a method of inhibiting GVHD in a mammal requiring DLI infusion in which donor engraftment with HSC occurs before LLME-treated donor lymphocytes are infused. Amended claim 5 recites a method of inhibiting GVHD wherein HSC is separated into CD34+ and CD34- fractions, the CD34- cells are treated with LLME to eliminate cytotoxic T cells, and then the two fractions are co-administered.

Rosenfeld, combined with Small and '112, does not teach or suggest all of the claimed elements of the present invention. First, Rosenfeld teaches treating bone marrow with LLME to eliminate cytotoxic T cells (see abstract) prior to transplantation and does not teach or suggest treating donor lymphocytes with LLME for infusion after allogeneic T cell-depleted hematopoietic stem cell transplantation, as claimed in amended claims 2 and 3 of the present application. Furthermore, as admitted by the Examiner and urged above, Rosenfeld does not teach the use of LLME-treated DLI after hematopoietic stem cell transplantation or the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, as claimed in claims 3 and 5 of the present application. Rosenfeld, Small and '112 do not mention or suggest all of these claim features. In addition, treatment of marrow with LLME by Rosenfeld caused high levels of toxicity of stem cells (see Table 2 and page 681, column 2, last paragraph, bridging page 682). Thus, Rosenfeld actually teaches away from using high doses of LLME as claimed in the present application, because their procedure causes stem cell toxicity.

Small does not correct the deficiencies of Rosenfeld, in that it does not teach or suggest the use of LLME to prepare T cell-depleted DLI for use in preventing GVHD following hematopoietic stem cell transplantation, nor does Small teach the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, as claimed in the present application.

In addition, '112 does not correct the deficiencies of Rosenfeld and Small. This reference teaches treating whole marrow with LLME prior to transplantation (column 4, lines 38-58; column 5, lines 4-9; column 6, lines 60-67), and does not teach treating donor lymphocytes for use after transplantation or separating $CD34^+$ cell from $CD34^-$ cells before treatment of $CD34^-$ cells with LLME, as claimed in the present application.

Therefore, Rosenfeld, Small, and '112, when combined, do not teach or suggest every element of the claims and thus cannot render the present invention prima facie obvious.

Furthermore, there would have been no motivation to combine these references, nor does the result of the purported combination teach or suggest all the elements of the claims. This is because, as discussed previously, none of these references, either alone or combined, teaches treating lymphocytes with LLME prior to infusion following hematopoietic stem

cell transplantation, or the separation of $CD34^+$ and $CD34^-$ cells before treatment of $CD34^-$ cells with LLME and infusing the cells after hematopoietic stem cell transplantation. On the contrary, Rosenfeld and '112 teach treating marrow with LLME. Thus, one of ordinary skill in the art would not have been motivated to combine Rosenfeld, which teaches LLME treatment of marrow, with Small, which teaches opportunistic infection and that low CD4 T cell counts correlate with an increased risk of infection. Nor would one of ordinary skill in the art been motivated to combine Rosenfeld and Small with '112, which teaches treating marrow with LLME because increased CD8 and NK cell counts increase the risk of GVHD.

Therefore, because there would have been no motivation to combine Rosenfeld, Small, and U.S. Patent No. 5,668,112, and because the combination does not teach or suggest every element of the claims as amended, the combination of these references does not render *prima facie* obvious the invention as claimed and the rejection of the claims under 35 U.S.C. § 103(a), should be withdrawn.

Conclusion

Based on the foregoing, all claims are believed to be in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,

ROBERT KORNGOLD

BY _____

DANIEL A. MONACO
Registration No. 30,480
DRINKER, BIDDLE & REATH, LLP.
One Logan Square
18th and Cherry Streets
Philadelphia, PA 19103
(215) 988-3312 ph.
(215) 988-2757 fax
Attorney for Applicant

Appendix A- "Marked-up" Version of Amended Claims as Required under 37 C.F.R. 1.121(c)(1)(ii)

2. (once amended) A method of inhibiting graft versus host disease in a mammal requiring donor lymphocyte infusion, said method comprising:

a) contacting the donor lymphocytes to be infused with an aqueous solution containing a therapeutically effective amount of L-leucyl-L-leucine methyl ester ex vivo;

b) selectively eliminating cytotoxic T-cells;

c) infusing said donor lymphocytes into said mammal; and

d) inhibiting graft versus host disease,

[The method of claim 1,]

wherein said mammal requires [DLI] donor lymphocyte infusion following allogeneic T cell-depleted [HSC] hematopoietic stem cell transplantation .

3. (once amended) A method of inhibiting graft versus host disease in a mammal requiring donor lymphocyte infusion, said method comprising:

a) contacting the donor lymphocytes to be infused with an aqueous solution containing a therapeutically effective amount of L-leucyl-L-leucine methyl ester ex vivo;

b) selectively eliminating cytotoxic T-cells;

c) infusing said donor lymphocytes into said mammal; and

d) inhibiting graft versus host disease,

[The method of Claim 1,]

wherein said infusing of said donor lymphocytes into said mammal occurs after donor [HSC] hematopoietic stem cell engraftment.

5. (once amended) A method of inhibiting [GVHD] graft versus host disease in a mammal requiring transplant of $CD34^+$ stem cells, said method comprising:

a) obtaining a preparation of hematopoietic stem cells from a mammal;

[a]b) separating the [HSC] hematopoietic stem cells to be infused into $CD34^+$ and $CD34^-$ fractions;

[b]c) contacting said $CD34^-$ [HSC] hematopoietic stem cell fraction with an aqueous solution containing a therapeutically effective amount of [LLME] L-leucyl-L-leucine methyl ester *ex vivo*;

[c]d) selectively eliminating [selective] cytotoxic T-cells in the $CD34^-$ [HSC] hematopoietic stem cell fraction;

[d]e) co-administering a therapeutically effective amount of said [LLME] L-leucyl-L-leucine methyl ester-treated $CD34^-$ [HSC] hematopoietic stem cell fraction with said $CD34^+$ [HSC] hematopoietic stem cell fraction; and

[e]f) inhibiting [GVHD] graft versus host disease.